DNA primases are essential for the initiation of DNA replication and progression of the replication fork. Recent phylogenetic analyses coupled with biochemical and structural studies have revealed that the arrangement of catalytic residues within the archaeal and eukaryotic primase has significant similarity to those of the Pol X family of DNA-repair polymerases. Furthermore, two additional groups of enzymes, the ligase/primase of the bacterial nonhomologous end-joining machinery and a putative replicase from an archaeal plasmid have shown striking functional and structural similarities to the core primase. The promiscuous nature of the archaeal primases suggests that these proteins might have additional roles in DNA repair in the archaea.

Subunit architecture of archaeal and eukaryotic primases

DNA primase has a pivotal role in DNA synthesis by making a short oligoribonucleotide that acts as a primer for DNA polymerase. Thus, the primase is essential for both leading and lagging strand synthesis. In principle the primase needs to act only on the leading strand, but is required for the initiation of every Okazaki fragment on the lagging strand. The DNA primase of eukaryotes and archaea is distinct in subunit composition, sequence and structure from the bacterial analogue, DnaG [1]. In contrast to monomeric DnaG, archaeal primases have two subunits [2], and in eukaryotes homologues of these two subunits further interact with two additional components to form the Pol α–primase assembly [1]. In this review, we shall refer to the two subunits shared by archaea and eukaryotes as the core primase and refer to the tetrameric eukaryotic assembly of core primase in complex with the B subunit and Pol α as the Pol α–primase (Table 1). The catalytic activity of the primase lies in the small subunit of the core primase [1]. Although the monomeric single catalytic subunit can be purified in recombinant form, interaction with the larger core primase subunits appears to both stabilize the catalytic subunit and modulate its activity to some degree. Perhaps the most dramatic example of this has come from studies of the primase of Pyrococcus species. Initial studies of this hyperthermophilic enzyme focused on the catalytic subunit in isolation [3]. Remarkably, this enzyme was capable of synthesizing long (up to 6 kb) DNA strands in the absence of ribonucleotides, that is, it could both initiate and extend DNA chains. The isolated enzyme had little or no ability to synthesize RNA. However, when the heterodimeric enzyme was reconstituted it showed very different properties: the DNA polymerase activity was substantially reduced and the RNA synthesis significantly stimulated [2]. An intriguing implication of this observation is that the large subunit might have the ability to modulate both processivity and substrate choice of the catalytic subunit. The mechanism by which this influence is exerted is currently unknown. The highly promiscuous nature of the archaeal primase is not restricted to the Pyrococcus enzyme; recent studies have revealed that the primase from the highly diverged archaeon Sulfolobus solfataricus also has the ability to initiate and extend both RNA and DNA chains for up to 1 kb or 7 kb, respectively [4].

In eukaryotes, the core primase synthesizes a short oligoribonucleotide primer (between 6 and 15 nt long, depending on the species studied), which is then extended by DNA synthesized by the Pol α component of the Pol α–primase complex [1]. In this light, it is tempting to speculate that the dual RNA and DNA synthesis capabilities of archaeal primases could fulfil primer synthesis and DNA extension functions, respectively. However, there is currently no evidence for this hypothesis. Indeed, experiments from the Ishino laboratory indicate that primers synthesized by archaeal primase are extended by the replicative polymerase in a reconstituted in vitro system [2]. Although archaeal primase has the capacity to make RNA primers in vivo, two pieces of evidence cast doubt on the in vivo significance of the property. First, it has been established that archaeal Okazaki fragments have RNA at their 5' end [5], and second, measurement of the apparent $K_m$ of the Sulfolobus primase for nucleoside triphosphates (NTPs) and deoxy-nucleoside triphosphates (dNTPs) reveals that the affinity for dNTPs is at least three orders of magnitude lower than that for NTPs [4]. In addition to possessing primase and polymerase activities, the Sulfolobus enzyme has recently been demonstrated to have 3' nucleotidyl terminal transferase activity [4,6].

Table 1. The subunit architecture of primase and primase/pol α from representative archaea and eukaryotes

<table>
<thead>
<tr>
<th></th>
<th>Eukaryotes</th>
<th>Archaea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human</td>
<td>S. cerevisiae</td>
</tr>
<tr>
<td>Pol α</td>
<td>p165</td>
<td>Po1 (180 kDa)</td>
</tr>
<tr>
<td>B subunit</td>
<td>p77</td>
<td>Po12 (79 kDa)</td>
</tr>
<tr>
<td>Primase</td>
<td>p50</td>
<td>Pri1 (48 kDa)</td>
</tr>
<tr>
<td>small</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primase</td>
<td>p59</td>
<td>Pri2 (58 kDa)</td>
</tr>
<tr>
<td>large</td>
<td></td>
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</tr>
</tbody>
</table>

*The names of the genes encoding the subunits are listed and the size of the protein products are indicated.
Structural studies of archaeal primases

To date, crystal structures for the isolated catalytic subunit of archaeal primases from *P. horikoshii* and *P. furiosus* have been reported [7,8]. The crystallographic analysis showed that the catalytic subunit folds in a novel tertiary structure made up of two domains: a larger, mixed α/β domain, where the catalytic activity resides (prim domain), and a smaller α-helical domain of unknown function (Figure 1). Inserted onto the fold of the catalytic domain is a zinc-binding motif, which represents a conserved feature of archaeal and eukaryotic primases. The functional role of the zinc-binding motif is not yet known. Intriguingly, bacterial primases also contain a zinc-binding domain that has been implicated in binding single-stranded DNA [1,9]. It is, therefore, tempting to speculate that the zinc-binding motif in archaeal and eukaryotic primases might have an analogous role. However, it should be noted that, in contrast to the situation with the archaeal primase, the bacterial primase zinc-binding site lies in a domain distinct from the catalytic site.

Although the archaeal/eukaryotic primase catalytic subunit fold is novel, some features of the active site, as gleaned from the available structural data, suggest that the mode of catalysis is likely to be similar to the general mechanism of enzymatic synthesis of both RNA and DNA proposed earlier [10]. Thus, a triad of aspartate residues, necessary for catalytic activity and invariant across archaeal and eukaryotic primases, can be superimposed on the catalytic aspartates of the human DNA polymerase β [11], a member of the X family of polymerases (Figure 1 and below). It therefore appears that convergent evolution has driven primases to adopt the same mechanism of catalysis, involving two divalent metal ions, proposed for other DNA and RNA polymerases [10]. The position of the active site was confirmed by diffusion of uridine 5′-triphosphate (UTP) in the *P. horikoshii* primase crystals [8]. The current model of primase catalysis postulates the existence of a rate-determining step when the enzyme is simultaneously bound to two nucleotides [1]. However, the structure of the primase–UTP complex provided no insight into the putative second nucleotide-binding event, which might therefore depend on the presence of the DNA template, or on the processing of the first nucleotide.

 Relatives of primase

The family of archaeal/eukaryotic primase-related molecules has recently been extended with the characterization of the ORF904 product of the pRN1 plasmid from the hyperthermophilic archaeon *Sulfolobus islandicus* (Figure 2a). This large protein has multiple functional domains and possesses ATPase, primase and polymerase activity [12]. The primase and polymerase activities are found in the N-terminal domain of the protein and the ATPase activity is associated with a putative helicase domain in the C-terminal region of ORF904. Initial studies classified ORF904 as a member of a novel family of DNA polymerases, family E. Interestingly, the biochemical properties of ORF904 are reminiscent of the catalytic subunit of archaeal primase. More specifically, the primase can initiate both RNA- and DNA-strand synthesis, with a preference for DNA, and in the presence of dNTPs can extend DNA strands for several kilobases [12]. Although the ORF904 primase/polymerase domain shows little primary sequence homology with other nucleo
acid polymerizing enzymes, the recent elucidation of the crystal structure of this domain of ORF904 has revealed striking structural parallels with the architecture of the catalytic centre of the \textit{Pyrococcus} archaeal primase \cite{7,13}. In particular, the arrangement of metal coordinating acidic residues shows tight conservation within a region of \(\beta\) sheet. Both ORF904 and \textit{Pyrococcus} enzymes have zinc-binding structural elements adjacent to the active centre of the enzyme. Surprisingly, however, these motifs are found in unrelated positions in the two enzymes (Figure 2b). Although it has been demonstrated that a positively charged cleft adjacent to the zinc-binding stem in ORF904 is important for template binding \cite{13}, it is not known what function the \textit{Pyrococcus} zinc-binding motif performs. However, this observation suggests that ORF904 and archaeal/eukaryotic primase are derived from a common ancestor, and further implies that this common ancestor lacked a zinc-binding domain and that two separate insertion events have occurred and been selected for during the evolution of the ORF904 and primase families.

\textbf{Bacterial mobile genetic elements encoding archaeal/eukaryotic primase proteins}

The bacteriophage T7 encodes a protein that has an N-terminal domain related to the bacterial primase DnaG and a C-terminal helicase domain related to the bacterial replicative helicase DnaB \cite{1}. A recent bioinformatics study has described the identification of a novel primase-helicase molecule found in an integrated phage in the bacterium \textit{Bacillus cereus} (Figure 3). Remarkably, the primase and helicase domains show homology not to bacterial DnaG and DnaB, but rather to the archaeal and eukaryotic primase catalytic subunit and the mini chromosome maintenance (MCM) presumptive replicative helicase \cite{14}. Although the primase–MCM fusion is unique to this particular phage, it is interesting to note that the primase-like domain is found in several other contexts in other bacterial genomes. More specifically, as shown in Figure 3, \textit{Rhodopirellula baltica} encodes an open reading frame (ORF) with high similarity to the \textit{B. cereus} primase domain (BLAST score of \(2 \times 10^{-9}\)). In \textit{R. baltica}, this ORF encodes only the primase molecule. However, the gene encoding the primase lies immediately upstream of one of two \textit{R. baltica} homologues of the helicase DnaB. Furthermore, this \textit{dnaB} homologue appears to be related to the subfamily of phage DnaB-like helicases (Figure 3).

Additionally, in \textit{Bdellovibrio bacteriovorus} HD100, a homologue of the \textit{B. cereus} phage primase domain is found in the N-terminal domain of a large ORF that has homology to bacterial retron reverse transcriptases. With the ever-increasing number of bacterial genome sequences it will be interesting to determine how ubiquitous the primase domain is. The fact that thus far all three homologues appear associated with mobile genetic elements or phage strongly implies that this could be a broadly distributed molecule.

\textbf{The primase–Pol X connection}

The first hint that there could be an architectural relationship between the Pol X family of DNA polymerases and primases emerged from sequence comparisons of a range of eukaryotic DNA Pol Xs and the small subunit of archaeal and eukaryotic primases \cite{15}. With the elucidation of the structure of human DNA Pol \(\beta\) and the first crystal structure of an archaeal primase, it became apparent that the primary sequence similarity was mirrored by a degree of structural similarity \cite{7,11}. More specifically, as mentioned above, the positioning of the highly conserved catalytic aspartate residues of archaeal primase was superimposable on the catalytic core of DNA Pol \(\beta\). Interestingly, however, the secondary structural contexts in which the aspartates are found are dissimilar between primase and DNA Pol \(\beta\), suggesting convergent evolution. Because these aspartates are also structurally conserved among polymerases and are implicated in the two-metal-ion mechanism of DNA polymerization \cite{10}, Kirk and Kuchta suggested that the archaeal primase might possess similar catalytic mechanisms to the family X polymerases \cite{15}. To date, five Pol X family members have been described in mammalian cells: Pol \(\beta\), Pol \(\lambda\), Pol \(\mu\), Pol \(\sigma\) and terminal
experiments have shown that, like archaeal primase, LigD is the eukaryotic primase catalytic subunit. Further, biochemical studies of this protein have revealed homology with the archaeal/eukaryotic primases, both enzymes possess template-dependent and template-independent polymerase activities [22,23] and can synthesize DNA de novo [17]. Additionally, these Pol X family members are prone to frameshift synthesis [24] and can fill in short gaps [19], supporting a role in NHEJ. Finally, Pol λ has a dRP lyase activity used for BER in vitro although its role in this process in vivo is still unclear [25].

Additional primase-like molecules in DNA repair

A further extension to the family of primase-like molecules has been made with the exciting discovery of a pathway for NHEJ in prokaryotes. Initial bioinformatics studies by the Koonin, Jackson and Doherty laboratories revealing the presence of prokaryotic homologues of the essential NHEJ factor, Ku [26,27], have been extended with a series of biochemical and genetic analyses, primarily focusing on bacterial organisms encoding this pathway [28–30]. In contrast to the highly complicated eukaryotic NHEJ machinery, the prokaryotic apparatus appears to require just two gene products [28]. One is the homologue of Ku, and the other has been named as LigD. Intriguingly, like the ORF904 protein described above, LigD is a multifunctional enzyme. In the case of LigD, the enzyme has ligase, polymerase, nuclease and primase activities [28,30]. Significantly, examination of the sequence of this protein has revealed homology with the archaeal/eukaryotic primase catalytic subunit. Further, biochemical experiments have shown that, like archaeal primase, LigD can synthesize RNA primers, has DNA-dependent DNA and RNA polymerase activity and, like Sulfolobus, it has 3′-nucleotidyl terminal transferase activity [28,31].

Concluding remarks

Thus, the archaeal/eukaryotic primases and a range of repair and mobile genetic element DNA polymerases are clearly related in both the arrangement of the catalytic aspartates within the active centre of the enzymes and by a common promiscuity in the reactions they can carry out. It appears therefore that this arrangement of the catalytic site has a degree of functional malleability that is ideally suited to performing nucleic acid synthetic functions on a diverse range of substrates. This adaptability could be significant in the context of the broad range of damaged DNA substrates that are dealt with by the repair polymerases. Interestingly, the archael primases appear to be capable of a broader spectrum of activities than their eukaryotic primase counterparts.

In light of the absence of members of the DNA-Pol-X family in archael species (with the exception of a single archael species, Methanobacterium thermoautotrophicum), it is tempting to speculate that the archael primases might have dual functionality, working both as bona fide primases but also playing roles in DNA-repair processes. Whether the relative efficiencies of the archael primases in DNA and RNA synthesis could be modulated by interaction with as yet unidentified factors remains an open question. If the archael primases also act as repair polymerases, then it is tempting to speculate that as eukaryotic cells evolved, the primase became dedicated to DNA replication and novel polymerases (perhaps co-opted from mobile genetic elements) adopted specific roles in DNA repair.

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